AD_____

Award Number: DAMD17-01-1-0349

TITLE: A Population-Based Investigation of the Role of Focal Adhesion Kinase (FAK) and E-Cadherin Expression in Breast Cancer Promotion, Progression, and Therapeutic Response

PRINCIPAL INVESTIGATOR: Sandra L. Deming

CONTRACTING ORGANIZATION: University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599-1350

REPORT DATE: October 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2004	Annual Summary (1 Oct 2003 - 30 Sep 2004)

4. TITLE AND SUBTITLE
A Population-Based Investigation of the Role of Focal Adhesion Kinase (FAK) and E-Cadherin Expression in Breast Cancer Promotion, Progression, and Therapeutic Response

5. FUNDING NUMBERS
DAMD17-01-1-0349

6. AUTHOR(S)
Sandra L. Deming

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599-1350

E-Mail: deming@email.unc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)
Presented within this final report are the results of the grant entitled "A population based investigation of the role of Focal Adhesion Kinase (FAK) and Ecadherin expression in breast cancer promotion, progression, and therapeutic response". In regards to the specific aims of the grant, immunohistochemical staining has been completed for both FAK and E-cadherin expression. Furthermore, scoring and analysis has been completed on 629 of the samples stained for FAK and 572 samples stained for e-cadherin, providing information on the prevalence of FAK and e-cadherin expression as well as their association with other known risk and prognostic factors in breast cancer. Data collection for vital status and cause of death is complete, as is the analysis addressing the roles of FAK and ecadherin expression in breast-cancer specific survival. The data suggest that neither FAK expression nor e-cadherin expression is significantly associated with stage of disease. In terms of 5-year survival, FAK, but not e-cadherin, expression is a significant prognostic indictor of breast cancer specific survival.

14. SUBJECT TERMS
No subject terms provided.

15. NUMBER OF PAGES
15

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Cover.....1

SF 298.....2

Table of Contents.....3

Introduction.....4

Body.....4-11

Key Research Accomplishments.....11

Reportable Outcomes.....12

Conclusions.....12-13

References.....-

Appendices.....14-15

Introduction:

One of the current problems in breast cancer diagnosis and treatment is the lack of verifiable markers indicative of prognosis, and ultimately therapeutic response. This is particularly important since many breast tumors are histologically similar, yet behave quite differently. By identifying molecular markers that are early indicators of more aggressive disease, we can better tailor treatments to achieve optimal results. Our study proposed to investigate the role of two such markers: E-cadherin and Focal Adhesion Kinase (FAK) as tenable prognostic markers in breast cancer. To address this question, tumor tissue from phase I cases of the Carolina Breast Cancer Study (CBCS) have been immunohistochemically stained for E-cadherin and FAK expression. CBCS is a population-based study in North Carolina that includes nearly 800 cancer cases collected from 1993-96. Extensive demographic information as well as medical, exposure, and work histories are available for the participants, which will allow for the evaluation of the independent role of E-cadherin and FAK in the context of known risk and prognostic factors. Determining the potential for FAK and E-cadherin to identify breast tumors requiring more aggressive treatment at an early stage, could ultimately increase survival and reduce breast cancer mortality.

Body:

Work progress with respect to the initial aims of the grant:

Aim 1:
a. Stain invasive tumor tissue sections with antibodies against FAK and E-cadherin (months 1-6)
b. Quantify immunohistochemical staining results (mos. 7-10)

Progress:
a. Both FAK and E-cadherin immunohistochemical staining have been completed for the phase I cases as proposed.
b. Quantification is in progress for FAK stained tissue (complete *n* =629)

Aim 2:
a. Statistically analyze the association between expression levels and invasive disease (month 11)
b. Determine the correlation between expression levels and stage of disease (month 12)

Progress:
a. Analysis has been completed for FAK and e-cadherin stained and quantified tissue (FAK:*n* = 629, e-cadherin *n* = 572)
b. Analysis has been completed for FAK and e-cadherin stained and quantified tissue (FAK:*n* = 629, e-cadherin *n* =572)

Aim 3:
a. Stratify women based on race and statistically determine if expression levels vary between African American and white breast cancer patients (month 13)
b. Stratify women based on age and statistically

determine if expression levels vary between younger and older breast cancer patients (month 14)

Progress: a. Analysis complete for both FAK and e-cadherin
b. Analysis complete for both FAK and e-cadherin

Aim 4: a. Obtain updated treatment histories for participants (months 15-27)
b. Ascertain current disease status, including recurrence and survival information for participants (months 28-30)
c. Statistically analyze the data to evaluate the association between FAK and E-cadherin expression levels and therapeutic response and survival (months 31-36)

Progress: a. Treatment information collection ongoing
b. Vital status and cause of death information collection complete.
c. Analysis between FAK expression, e-cadherin expression, and breast cancer specific survival is complete.

Results as of October 15, 2004: FAK

Please note, the data and results presented in the document are and unpublished, and thus should be protected accordingly.

All of the results presented are based upon cases from entire Phase I of the Carolina Breast Cancer Study for whom tissue had been immunohistochemically stained for FAK expression. The stained tissues from this sample were the first to be scored and analyzed for this data.

Table 1 presents the characteristics of the patients is this dataset. The cases in our dataset were a mean of 48.5 years of age and presented with a full range of stage of disease at time of diagnosis, which facilitated complete analysis by stage.

Table 1. Characteristics of the patients with tissue stained for FAK

N = 188	
Age (years)	
Mean	48.5
Median (Range)	47.0 (25-75)
Race *n* (%)	
African American	261 (41.5%)
Non-African American	368 (58.5%)
Stage *n* (%)	
1	76 (42.9%)
2	82 (46.3%)
3+4	19 (10.7%)
Missing	297

Our first analysis involved determining the prevalence and pattern of FAK expression in our sample of invasive tumors. The

characteristics of FAK expression, including percent tumors positive, intensity, and percent cells positive can be found in table 2.

Table 2. Characteristics of FAK expression in breast tumors

N = 629	
FAK expression (+/-)	
Positive	154 (24.5%)
Negative	425 (75.5%)

One of the goals of our grant proposal was to evaluate the correlation between FAK expression and severity of disease. Table 3 summarizes the prevalence and pattern of FAK expression in tumors stratified by stage at diagnosis. These data suggest that FAK expression is similar across all stages of disease at diagnosis, leading to the conclusion that FAK expression is not associated with stage.

Table 3. FAK expression by stage

N = 629		
FAK expression (+/-)	% Positive	
Stage 1	19.8	
Stage 2	25.7	Chi sq P=0.29
Stage 3 +4	30.0	
Stage 4	24.5	

Additionally we were interested in determining the association between FAK expression and invasive disease to ultimately determine if FAK expression provides information independent of currently known risk and prognostic factors. The following table (4) presents the association between FAK expression and other known risk factors in our sample. To determine in FAK expression was associated with age at diagnosis, we evaluated FAK expression by age. There was no difference across the three categories of age groups suggesting that FAK expression is not associated with age. These data suggest that FAK positive tumors are strongly associated with HER2+ tumors and with P53+ tumors. Additionally, FAK expression is inversely associated with both ER+ and PR+ tumors. Furthermore, positive FAK expression is strongly associated with tumors with a high mitotic index, high nuclear grade, and well or moderately differentiated histologic grade tumors.

Table 4: Association of FAK expression with known risk factors.

Risk Factor	**FAK Positive**	**FAK Negative**	**OR (95% CI)**	**Chisq p**
Age				
<40 years	94	42		P=0.09
40-50 years	183	58		
>=50 years	190	50		
P53 Status				
P53+	91	201	2.5(1.7-3.3)	P=0.0002
P53-	60	270		
HER2 Status				
HER2+	49	96	2.0 (1.4-3.3)	P=0.003
HER2-	105	376		

Estrogen Receptor				
ER+	71	284	0.4(0.3-0.6)	P=0.002
ER-	78	175		
Progesterone Receptor				
PR+	65	275	0.4(0.3-0.6)	P=0.0003
PR-	83	178		
Mitotic index				
High (MI>10)	59	290	0.4(0.3-0.5)	P=0.0001
Low (MI<=10)	93	180		
Histologic Grade				
Well-mod differentiated	44	173	0.6(0.4-0.8)	P=0.008
Poorly differentiated	109	299		
Nuclear Grade				
Slight-mod. Pleomorphism	96	165	4.9(3.3-7.3)	P=0.0001
Marked pleomorphism	57	308		

In terms of FAK protein expression and breast cancer survival, 5-year breast-cancer specific survival was evaluated. Data collected from the National Death Index was evaluated to confirm mortality due specifically to breast cancer. These deaths were included in the survival analysis to reduce bias due to misclassification of cause of death. Figure 1 (Attached in appendix) is the 5-year Kaplan Meier survival curve based on FAK expression. Survival analysis suggests that tumors negative for FAK expression have higher 5-year breast cancer specific survival (79.2% for FAK+ vs. 88.0% for FAK- tumors). Cox Proportional Hazards analysis was utilized to determine the independent prognostic value of FAK expression after adjusting for known prognostic factors.

Table 5: Cox proportional hazards ratios for breast cancer specific survival by FAK protein expression.

N = 629	**Hazard Ratio***	**95% confidence interval**
FAK positive	1.7	(1.1 - 2.9)
FAK negative	1.0	

* HR adjusted for estrogen receptor, progesterone receptor, stage, p53 expression, mitotic index, race, her2 expression, histologic grade, and nuclear grade.

Cox proportional hazards modeling suggests that FAK does have independent prognostic value after controlling for many of the currently accepted prognostic factors.

E-cadherin expression

All of the results presented are based upon cases from the entire Phase I of the Carolina Breast Cancer Study for whom tissue had been immunohistochemically stained for e-cadherin expression. A scoring system was developed and tested for e-cadherin intra- and inter-rater reliability using kappa statistics (Data not shown). Kappa statistics indicated that reliability was in the good to excellent range, providing confidence in the consistency of scoring.

Table 6 presents the distribution of e-cadherin expression in the CBCS phase I cases.

Table 6. Characteristics of e-cadherin expression in breast tumors

N = 572	
E-cadherin expression(+/-)	
Positive	214 (37.4%)
Negative	358 (62.6%)

Table 7 presents the characteristics of the patients is this dataset based upon e-cadherin protein expression. The total number of tumor evaluated for e-cadherin protein expression was 572 cases.

Table 7: Distribution of e-cadherin expression by various demographic and tumor characteristics among CBCS participants (continued)

	e-cadherin positive cases (n=358)	**%**	**e-cadherin negative cases (n=214)**	**%**	**p-value[a]**
Age					
<40	76	21.2	49	22.9	p=0.83
40-50	145	40.5	88	41.1	
>50	137	38.3	77	36.0	
Missing=0					
Race					
Non African American	212	59.2	127	40.7	p=0.98
African American	146	40.8	87	59.3	
Missing=0					
Stage					
I	136	41.1	68	34.5	p=0.42
II	162	49.0	103	52.3	
III	25	7.6	20	10.2	
IV	8	2.4	6	3.0	
Missing=73	27		17		
Estrogen receptor					
Positive	136	39.4	97	47.1	p=0.08
Negative	209	60.0	109	52.9	
Missing=69			8		
Progesterone receptor					
Positive	136	39.8	103	50.0	p=0.02
Negative	206	60.2	103	50.0	
Missing=79	16		8		
Menopausal status					
Pre/Peri	188	52.5	118	55.1	p=0.54
Post	170	47.5	96	44.9	
Missing=0					
BMI					
<=24.99 kg/m^2	136	38.9	7	32.5	p=0.70
25.00-29.99 kg/m^2	98	28.0	74	31.5	
>=30.00 kg/m^2	116	33.1	64	36.0	

Family history of breast or ovarian cancer					
Yes	44	12.6	33	15.9	p=0.28
No	306	87.4	175	84.1	
Missing=0					
Age at Menarche					
<13	175	48.9	115	53.7	p=0.26
>=13	183	51.1	99	46.3	
Missing=0					
Parity					
Nulliparous	56	15.6	29	13.6	p=0.77
1 child	64	17.9	45	21.0	
2 children	111	31.0	66	30.8	
3 or more children	127	35.5	74	34.6	
Missing=0					
Ever lactated					
No	238	66.5	148	69.2	p=0.51
Yes	120	33.5	66	30.8	
Missing=0					
Oral Contraceptive use					
Ever	222	62.2	150	70.4	p=0.05
Never	135	37.8	63	29.6	
Missing=2	1		1		
Duration of OC use					
Never	115	32.2	55	25.8	p=0.40
1-12 months	67	18.8	40	18.8	
13-60 months	75	21.0	44	20.7	
61-120 months	59	16.5	41	19.2	
121 months or greater	41	11.5	33	15.5	
Missing=0					
Hormone replacement therapy use[b]					
Ever	60	35.3	51	53.1	p=0.06
Never	110	69.7	45	46.9	
Missing=0					
Alcohol use					
Ever	252	70.4	158	73.8	p=0.38
Never	106	29.6	56	26.2	
Missing=0					
P53 functional mutation					
Positive	180	49.7	111	51.9	p=0.71
Negative	178	50.3	103	48.1	
Missing=0					

[a] p-values for test of differences in proportions based on Cochran-Mantel-Haentzel chi-square statistics.

[b] Among post=menopausal women

Based on these data, e-cadherin expression is significantly inversely associated with progesterone receptor and oral contraceptive

use. None of the other risk factors evaluated differed significantly in terms of e-cadherin expression distribution.

Table 8 below presents Odds ratios for e-cadherin + e-cadherin negative breast cancer based on various known and suspected risk factors for breast cancer. Of the factors investigated, only oral contraceptive use was significantly associated with e-cadherin expression breast tumors as participants ever using oral contraceptives were 1.8 times more likely than those never using oral contraceptives to have e-cadherin positive versus negative breast cancer after adjusting for age, race and sampling fractions. This would suggest that oral contraceptive use is protective against, e-cadherin breast cancer, a type of breast cancer thought to be more associated with increased likelihood of metastasis.

Table 8: Odds ratios and 95% confidence intervals for e-cadherin + versus e-cadherin - in CBCS

	E-cadherin + vs. E-cadherin - cases OR (95% CI)[a]
Menopausal status	
Pre/Peri	1.0
Post	1.0 (0.6 - 1.7)
BMI	
<=24.99 kg/m^2	1.0
25.00-29.99 kg/m^2	1.3 (0.9 - 2.1)
>=30.00 kg/m^2	1.2 (0.8 - 1.8)
Family history of breast or ovarian cancer	
Yes	1.5 (0.9 - 2.4)
No	1.0
Age at Menarche	
<13	1.3 (0.9 - 1.8)
>=13	1.0
Parity	
Nulliparous	1.0
1 child	1.6 (0.9 - 2.9)
2 children	1.3 (0.8 - 2.3)
3 or more children	1.4 (0.8 - 2.4)
Oral Contraceptive use	
Ever	1.8 (1.2-1.8)
Never	1.0
Duration of OC use	
Never	1.0
1-12 months	1.7 (1.0 - 3.0)
13-60 months	1.6 (0.9 - 2.9)
61-120 months	1.7 (0.9 - 3.0)
121 months or greater	2.1 (1.1 - 4.1)
Hormone replacement therapy use[b]	
Ever	1.6 (0.9 - 2.7)
Never	1.0

[a] Odds ratios adjusted for age, race, and sampling fractions
[b] Among post-menopausal women only

5-year breast-cancer specific survival was evaluated based upon e-cadherin protein expression. Again, data collected from the National Death Index was evaluated to confirm mortality due specifically to breast cancer. These deaths were included in the survival analysis to reduce bias due to misclassification of cause of death. Figure 2 (Attached in appendix) is the 5-year Kaplan Meier survival curve based on e-cadherin expression. Survival analysis suggests that there is no difference in survival based on e-cadherin expression. 84.4% of patients with e-cadherin positive tumors and 85.5% of participants with e-cadherin negative tumors were alive at 5-years.

Various demographic and prognostic factors were evaluated for their associations with survival. Increasing stage, negative estrogen receptor, negative progesterone receptor, high mitotic index, poor histologic and nuclear grade, and P53 functional mutation positivity were all associated with decreased breast-cancer specific survival.

Cox Proportional Hazards analysis was utilized to adjust for these factors to thereby determine the independent prognostic value of e-cadherin expression. Results are found in table 9 and indicate that e-cadherin is not an independent prognostic factor in breast cancer specific survival.

Table 9: Cox proportional hazards ratios for breast cancer specific survival by FAK protein expression.

N = 572	Hazard Ratio*	95% confidence interval
e-cadherin positive	0.9	(0.5 - 1.5)
e-cadherin negative	1.0	

* Models adjusted for ER status, PR status, Her2 amplification, P53 functional mutation status, mitotic index, nuclear grade, and histologic grade.

Key Research Accomplishments:

There are a number of key research accomplishments for this project:

- Immunohistochemical staining of tumor tissue for E-cadherin expression is complete
- Immunohistochemical staining of tumor tissue for FAK expression is complete
- Scoring of FAK stained tissue is complete for 629 of the stained phase I cases.
- Scoring of e-cadherin stained tissue is complete for 572 phase I cases
- Data analysis for the FAK stained and scored samples has been preformed including determining associations with various demographic, risk, and prognostic factors.
- Data analysis for e-cadherin stained and scored tissue has been preformed determining associations with various demographic, risk, and prognostic factors.

- Vital status and cause of death information collected for phase I cases.
- Breast cancer specific survival analysis was completed for both FAK and e-cadherin tumor expression.
- Independent prognostic value of FAK and e-cadherin protein expression was determined.

Reportable Outcomes:

Based upon the results from this study on this project, an abstract and poster was presented at the 2002 Department of Defense Era of Hope Breast Cancer Meeting in Orlando Florida. The poster was titled:

The role of E-cadherin and FAK expression as prognostic markers in breast cancer progression and survival

Sandra L. Deming[1], Amy L. Lark[2], Lynn G. Dressler[2], Dave W. Cowan[2], William G. Cance[2], and Robert C. Millikan[1,2]

[1] Department of Epidemiology, School of Public Health
[2] Lineberger Comprehensive Cancer Center

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Conclusion: The conclusions based on the preliminary data indicate that the prevalence of FAK expression among invasive cases of Phase I of the Carolina Breast Cancer Study is 29.8%. FAK expression status was associated with positive P53 expression, positive Her2 expression, later stage at diagnosis, ER and PR negativity, high mitotic index, high nuclear grade (degree of pleomorphism), and lymph node metastasis. FAK expression was not associated with histologic grade (degree of differentiation). High FAK expression appears to be associated with known factors indicative of more aggressive disease and worse prognosis. Specifically, high FAK expressing tumors are 2.0 times more likely to have amplified Her2, 2.5 times more likely to be ER negative, 2.5 times more likely to be PR negative, and 2.5 times more likely to have high mitotic activity as compared to low expressers (all p-values < 0.05). In terms of survival as analyzed using Cox proportional hazards regression, FAK was determined to have independent prognostic value after adjusting for estrogen receptor status, progesterone receptor status, stage, P53 immunohistochemistry, mitotic index, race, Her2 expression, histologic grade, and nuclear grade. Individuals expressing FAK were 1.7 times more likely to die of breast cancer over a 5-year period as compared to those negative for FAK expression.

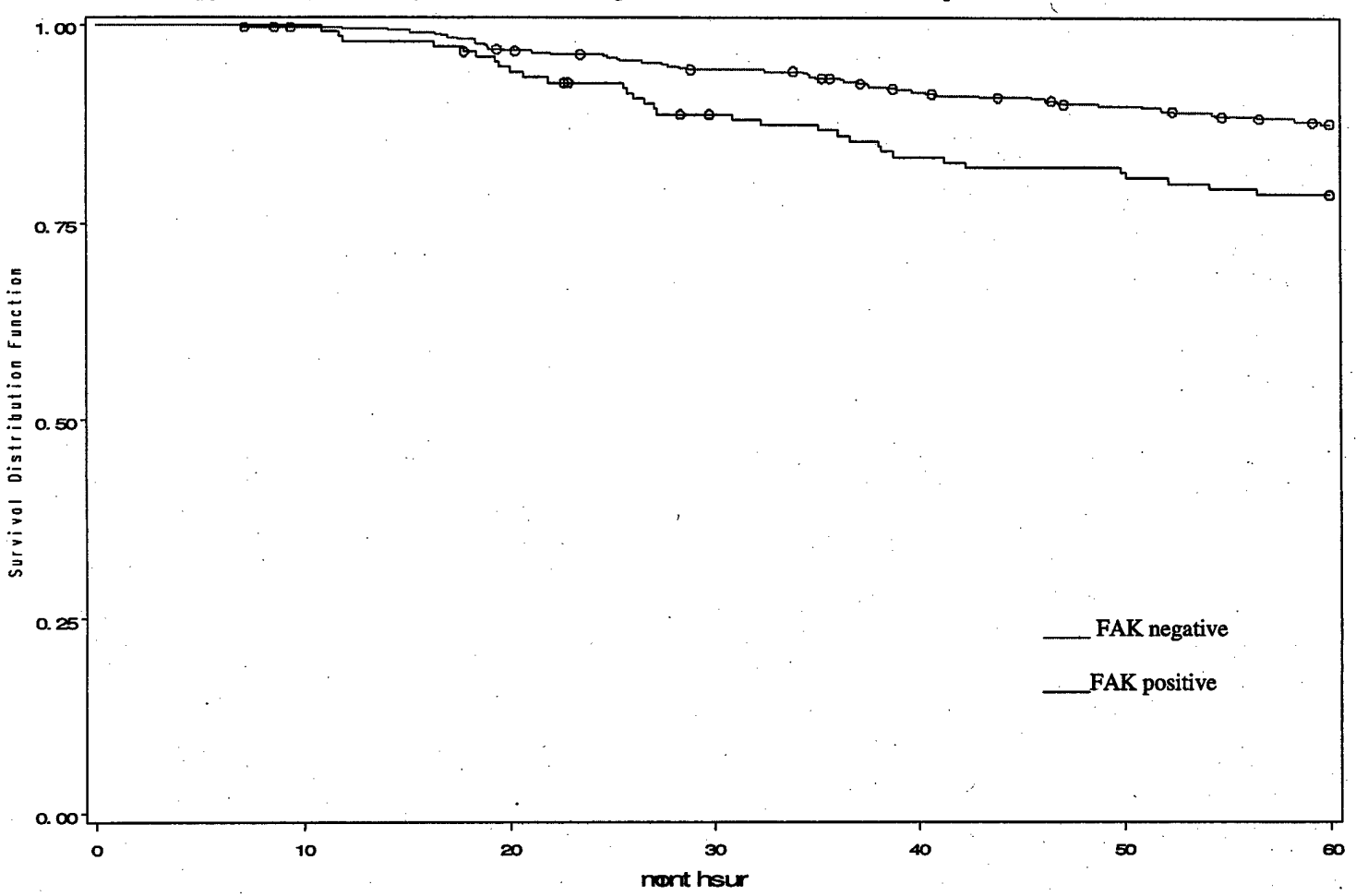
In terms of e-cadherin expression, the prevalence of immunohistochemical staining among CBCS phase I tumors was 62.6%. Of the demographic and risk factors evaluated, oral contraceptive use was significantly associated with e-cadherin tumor expression. Individuals ever using oral contraceptives were 1.8 times more likely to have e-cadherin positive tumors as compared to those never using after adjusting for age, race and sampling fractions. All other factors evaluated were not significantly associated with e-cadherin expression. These data are suggestive that oral contraceptive use may be protective again e-cadherin negative expression tumors, which are thought to have increased metastatic potential. Although decreased e-cadherin expression is thought to be associated with metastatic

potential, survival analysis demonstrated no difference in 5-year breast cancer-specific survival based upon e-cadherin expression. 84.4% of patients with e-cadherin positive tumors and 85.5% of participants with e-cadherin negative tumors were alive at 5-years. Cox proportional hazard analysis allowed for multi-variable analysis to adjust for known prognostic factors to determine e-cadherin's independent prognostic value. After adjusting for ER status, PR status, Her2 amplification, P53 functional mutation status, mitotic index, nuclear grade, and histologic grade, e-cadherin was determined not to have independent prognostic value.

It is also important to evaluate these results in relation to what is currently known in the breast cancer field. As mentioned, breast cancer is a very heterogeneous disease where tumors similar in phenotype behave quite differently. It was our desire to evaluate both FAK and e-cadherin as potential viable markers of progression, as well as identify factors that may be associated with increased risk of subsets of breast cancer defined by either FAK or e-cadherin expression. Evaluating risk factors in relation to breast cancer subsets defined by these markers may allow for identification of potential causes of disease that may go un-recognized when breast cancer is analyzed as a homogeneous disease. Most notable from this research is that oral contraceptive use appears to be protective against e-cadherin negative breast cancer, which is generally thought to have greater metastatic potential. This type of data becomes particularly important since oral contraceptive use is a modifiable exposure. In terms of survival, results of this study have ruled as e-cadherin as having independent prognostic information over currently utilized prognostic factors in breast cancer. Similar analyses indicate that FAX expression has independent prognostic value and suggest that tumors positive for FAK expression may require more aggressive therapy.

Appendix

Appendix Figure 1: 5-year breast cancer-specific survival based on FAK expression



Appendix Figure 2: 5-year breast cancer-specific survival by E-cadherin tumor expression status